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Research Brief

Evaluation of efficacy of 18 strains of entomopathogenic nematodes (Rhabditida) against *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) under laboratory conditions

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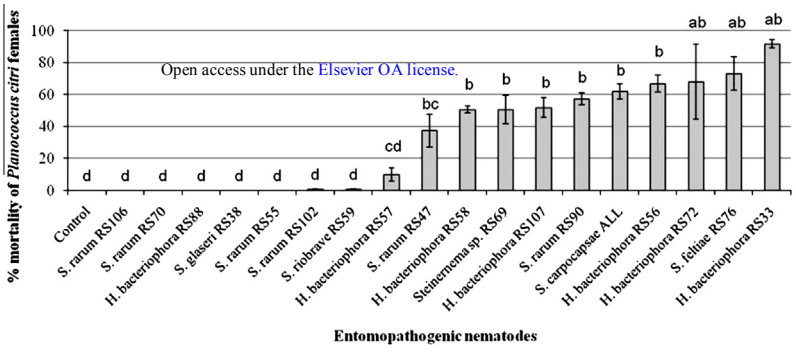
HIGHLIGHTS

- Entomopathogenic nematodes are pathogenic to *Planococcus citri*.
- *Heterorhabditis bacteriophora* RS33 are the most virulent nematode to *P. citri*.
- Entomopathogenic nematodes are potential agents to control *P. citri* in vineyards.

GRAPHICAL ABSTRACT

Screening of insect parasitc nematodes for the *Planococcus citri* control in grapevines.



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ABSTRACT

Planococcus citri (Risso, 1813) (Hemiptera: Pseudococcidae) is an important plant virus vector in grapevine crops in Brazil and other countries. The mealybug grows in roots and leaves of the grapes. Entomopathogenic nematodes (EPNs) are efficient control agents against insects associated to the soil and could be applied with the same equipment used for chemical insecticides. The aim of this study was to select effective EPNs for controlling *P. citri* females in laboratory conditions (25 ± 1 °C, UR 60 ± 10%). We tested 17 native [*Steinernema rarum* (6 strains), *Steinernema glaseri*, *Steinernema feltiae*, *Steinernema riobrave*, *Steinernema* sp., *Heterorhabditis bacteriophora* (7 strains)] and only one exotic strain (*Steinernema carpocapsae* ALL). The bioassays were done on Petri dishes infested with females of *P. citri*, which were sprayed with EPNs juveniles. The strain with larger pathogenicity and virulence in laboratory was *H. bacteriophora* RS33 (from 69.0% to 92.2% of mortality), native of Rio Grande do Sul.

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The mealybugs (Hemiptera: Pseudococcidae) are considered the main causal agent for virus spreading in vineyards [*Grapevine leafroll virus* 3 (GLRaV-3), *Grapevine virus A* (GVA) and *Grapevine virus B* (GVB)] in South Africa, Argentina, Australia, Chile, Spain, United States, Italy, New Zealand, Portugal and Uruguay (Walton and Pringle, 2004; Charles et al., 2006). These insect vectors cause death of plants in the field directly (damaging the fruit and causing appearance of sooty mold) and indirectly, by transmitting viruses. (Morandi Filho et al., 2009).

In Brazil, the main species of mealybug in vineyards is *Planococcus citri* (Risso, 1813) (Morandi Filho et al., 2009). This species develops on leaves and roots of grapes, making it difficult to be controlled. The most used form of control is treatment with lime sulfur (4°Bé), which is not effective for reducing high infestations. For this, a wait of 40 days should be required for using mineral or vegetal oils (Botton et al., 2003).

The use of entomopathogenic nematodes (EPNs) for *P. citri* control in the vineyards can be a viable strategy, because the environment taken by the mealybug, especially when it is on the roots, is quite similar to the one required by the EPNs. Because of this Andaló et al. (2004b) and Alves et al. (2009a) observed that *Heterorhabditis* spp. and *Steinernema* spp. caused high mortality of *Dysmicoccus texensis* females (Tinsley, 1900) (Hemiptera: Pseudococcidae) under laboratory conditions. Moreover, Alves et al. (2009b) found that Heterorhabditis sp (strains JPM3 and CCA) presented host seeking behavior in sand column, causing high mortality of the mealybug in laboratory, greenhouse and field, in levels equivalent to the chemical control. It is important to mention that *D. texensis* is, like *P. citri*, is associated to the soil, which reinforces the possibility of controlling these plagues with EPNS. This way, the aim of this work is to evaluate 10 strains of entomopathogenic nematodes against female *P. citri* under laboratory conditions. Entomopathogenic nematodes were isolated from different physiographic regions of Rio Grande do Sul, Brazil (Barbosa-Negrisoli et al., 2009), representing an important source of agents for biocontrol of pests occurring mainly in southern Brazil. In this study, some isolates were evaluated against *P. citri* in the laboratory, aiming future application for controlling this pest in vineyards.

The experiments were conducted at the laboratories of Federal University of Pelotas and Embrapa Clima Temperado, Rio Grande do Sul, Brazil. Initially, the mealybugs were collected in roots of grapevine crops, which were multiplied in pumpkin cultivar "Cabochá" (*Cucurbita maxima* Duchesne), maintained at room temperature (25 ± 3 °C) in plastic boxes (43 × 24 × 18 cm) covered with voile. When the pumpkins became unviable for feeding insects, due to its decomposition, new fruits were placed beside them, encouraging the migration of nymphs to the new substrate. Eighteen strains of entomopathogenic nematodes (EPNs) were used in the experiments, of which 17 were native [*Steinernema rarum* (RS47, RS55, RS70, RS90, RS102, RS106), *Steinernema glaseri* (RS38), *S. feltiae* (RS76), *S. riobrave* (RS59), *Steinernema* sp. (RS69), *Heterorhabditis bacteriophora* (RS33, RS56, RS57, RS58, RS72, RS88, RS107)], and for comparison purposes, an exotic strain (*Steinernema carpocapsae* ALL) was also tested (Table 1). The nematodes were produced in *Galleria mellonella* L. (Lepidoptera: Pyralidade) larvae as proposed by Kaya and Stock (1997) and stored in polyurethane sponge at 12 °C. *G. mellonella* larvae were reared on artificial diet, according to Parra (1998).

The first experiment aimed to select efficient EPNs strains against females of *P. citri*. The experimental arena consisted of Petri dishes (9 cm diameter, 1.5 cm height) containing 1 cm water agar layer (1%) covered by 2 mm layer of sterilized sand (125–250 μm). A piece of pumpkin shell (9 cm^2 by 1 cm height) was placed over the substrate in the Petri dish over which 40 *P. citri* females were released. Nematodes were spread using a spraying equipment (Potter-Precision Laboratory Spray Tower, Burkard Scientific Ltd, UK) calibrated at 10 lb/in^2 and 2.0 ± 0.1 mL of aqueous suspension per dish, at a concentration of 400 IJs [≈6.2 infective juveniles (IJs) per cm^2]. Then, the dishes were covered and incubated (25 ± 1 °C, UR 60 ± 10%) for four days. The experimental design was randomized, with 18 treatments (EPNs isolates) and the control (water). Data were submitted to ANOVA and compared by Tukey test at 5% probability.

Based on mortality rates obtained in the first experiment, three most virulent strains were selected to determine their lethal concentration (LC_{50} and LC_{90}) further on. The experimental method was similar to the description above, using the following concentrations: 0 (control), 400, 800, 1200, 1600 and 2000 IJs per Petri dish. The experimental design was random, with 3 treatments (EPNs isolates) and the control (sterile water). Mortality means were subjected to the Probit analysis to determine the lethal concentrations (PoloPlus 1.0, Probit and Logit Analysis, LeOra Software®).

A third bioassay was designed to evaluate the efficiency of *H. bacteriophora* RS33, selected as the most virulent, to spray on squash infested with *P. citri*. Pumpkins (10 cm diameter) were cut in half and placed in Petri dishes (15 cm diameter, 1.5 height) containing liquid paraffin (to avoid liquid accumulation). Afterwards, layers of water-agar and sand (as in the previous bioassay) were poured over the solidified paraffin. The application of nematodes was performed with hand pre-compression sprayer (PCP 424.00, Guarany®, 1L) at a pressure of about 10 lb/in^2, with a volume of 4.0 ± 0.1 mL of aqueous suspension per dish, at a concentration of 2.491 IJs per dish (≈14 IJs/cm^2), based on the lethal concentration (LC_{90}). The experimental design was randomized and each Petri dish was considered as a repetition, in a total of five replicates per treatment. The experimental design was randomized, with 2 treatments (EPN isolate) and the control (sterile water). Larvae mortality caused by infective juveniles in bioassays was confirmed by symptoms of infection (change in color and viscosity of the insect hemolymph caused by the presence of the nematode symbiotic bacteria) and/or the presence of nematodes after 96 h exposure. Data were submitted to ANOVA and compared by Tukey test at 5% probability.

Only 10 EPNs strains were pathogenic to females of *P. citri* in laboratory, from the 18 tested ($F = 21.0$; $P = 0.0001$, df = 18) (Fig. 1). Although no significant differences were found, we selected *H. bacteriophora* RS33, *Steinernema feltiae* RS76, *H. bacteriophora* RS7 that caused 92.2%, 73.2% and 68.2% mortality of *P. citri*, respectively. *H. bacteriophora* RS33 showed the highest virulence on the females of *P. citri*, presenting LC_{50} equal to 7.7 (1.07–11.9) and LC_{90} equal to 22.4 (15.6–57.1) IJs per insect. Strains *H. bacteriophora* RS72 and *S. feltiae* RS76 were the less virulent ones (Table 2). Based on these results, the strain *H. bacteriophora* RS33 was selected for the subsequent bioassay.

Results of the third assay confirmed efficiency of the isolated *H. bacteriophora* RS33 (69.0% mortality compared to control, with 9.4%) ($F = 342$, $P = 0.001$, df = 1), indicating these strains as the most promising ones for controlling *P. citri*.

The results obtained with *P. citri* in this study are similar to those observed by Andaló et al. (2004a), Alves et al. (2009b) and Alves and Moino (2009), that demonstrate the potential use of EPN to control Pseudococcidae and especially *D. texensis* in coffee crops. Future studies should be conducted to evaluate efficiency of *H. bacteriophora* RS33 in the field.

Moreover, there is a possibility to use a mixture of chemical insecticides and EPNs, as carried on by Andaló et al. (2004a). These authors assessed the compatibility of EPNs with pesticides used in coffee crops and concluded that viability and infectivity of IJs were not affected after exposition to some insecticides such as imidacloprid and thiamethoxam, normally used against *D. texensis* (Souza et al., 2007). Another possibility would be to mix nematodes with

Table 1
List of strains tested, season, vegetation, altitude, latitude and longitude from where the nematodes were isolated are given.

Isolated	Season	Covering vegetation	Soil type	UP[1]	Place	Altitude (m)	T (°C)[2]
Heterorhabditis bacteriophora RS 72	Summer	Wheat and tangerine	Latosol	PM	Rosário do Sul	122	24
Heterorhabditis bacteriophora RS 88	Summer	Orange and passimon	Latosol	PM	Julio de Castilhos	380	22
Heterorhabditis bacteriophora RS 33	Spring	Strawberry and peas	Neosol	PL	Capão do Leão	9	20
Heterorhabditis bacteriophora RS 56	Winter	Soybeans and barley	Latosol	PM	Bom Jesus	899	18
Heterorhabditis bacteriophora RS 57	Winter	Forest	Latosol	PM	Lagoa Vermelha	825	19
Heterorhabditis bacteriophora RS 58	Winter	Corn	Vertisol	DP	Lagoa Vermelha	825	19
Heterorhabditis bacteriophora RS107	Summer	Eucaliptus sp.	Neosol	PM	Arroio Grande	177	25
Steinernema carpocapsae ALL	-	-	-	-	Georgia, USA	-	-
S. feltiae RS 76	Summer	Corn	Latosol	PM	Cacequi	133	28
S. riobrave RS 59	Winter	Native field	Latosol	PS	Lagoa Vermelha	827	18
Steinernema glaseri RS 38	Winter	Corn	Neosol	PM	Passo Fundo	647	18
Steinernema rarum RS 47	Winter	Corn, field and forest	Latosol	PM	Planalto	680	19
Steinernema rarum RS 55	Winter	Native field	Latosol	PM	Muitos Capões	889	17
Steinernema rarum RS 70	Summer	Wheat	Neosol	PC	Dom Pedrito	147	24
Steinernema rarum RS 90	Summer	Native field	Neosol	PM	Canguçu	390	22
Steinernema rarum RS102	Summer	Tobacco	Neosol	PL	São José do Herval	680	23
Steinernema rarum RS106	Summer	Pinus sp.	Neosol	PM	Cidreira	3	23
Steinernema sp. RS 69	Summer	Soybean tillage	Alisol	DP	Dom Pedrito	137	24

[a] UP = units of landscape; PL = plain coastal lagoon; PM = median plateau; DP = central depression; PS = south plateau; PC = central plateau.
[b] *T* = soil temperatures in Celsius.

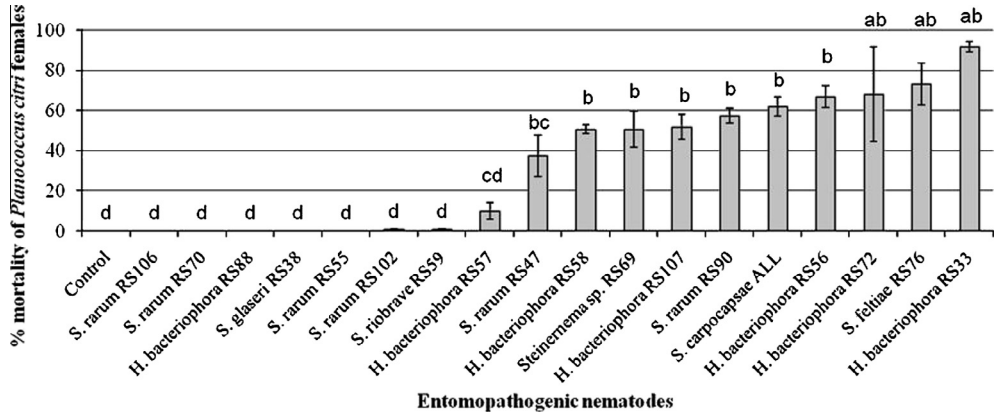

Fig. 1. Mortality (±SE) of *Planococcus citri* females 96 h after exposure to different strains of entomopathogenic nematodes in the laboratory (25 ± 1 °C, UR 60 ± 10%).

Table 2
Probit analysis to determine the lethal concentration (LC_{50}, LC_{90}) of *Heterorhabditis bacteriophora* RS33, *Heterorhabditis bacteriophora* RS72 and *Steinernema feltiae* RS76 against *Planococcus citri* females.

EPN	LC50	Conf.[a]	LC90	Conf.	Slope	X[b]	Hetero2
H. bact. RS33	7.7	1.07–11.9	22.42	15.6–57.1	2.7	275	1.38
H. bact. RS72	51.2	38.7–91.6	353.5	159.5–1445	1.5	10	0.547
S. feltiae RS76	49.7	42.1–65.9	131.4	905–261.0	3.4	04	0.2

[a] Confidence interval.
[b] Heterogeneity (considering data fitting when heterogeneity is lower than 4, despite elevated X^2).

other types of pesticides, such as fungicides (Laznik et al., 2012) or other (chemical and/or biological) products which could be used simultaneously, reducing costs of phytosanitary control (Negrisoli et al., 2010). This way, Morandi Filho et al. (2009), when evaluating the effect of growth regulators and neonicotinoids against *P. citri*, showed that acetamiprid, imidaclorpid and thiamethoxam caused 82%, 94% and 82% of mortality in grapevine, respectively. This demonstrates that the combination of two methods, in this case, biological and chemical controls, can be an effective alternative to significantly reduce the population of these insects in coffee and grape crops.

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